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Effects of rumen bacterial lipases on ruminal lipid metabolism

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Introduction

The burden of chronic, fat-related diseases upon the NHS is reaching a critical level and can be linked to an increased intake of saturated fats (SFA). SFAs are found in many products, including red meat and milk despite the ruminant diet being rich in health-beneficial poly-unsaturated fatty acids[1,2]. This is due to lipolysis and biohydrogenation (fig 1), processes that saturate PUFA in the rumen to protect bacteria from PUFA's toxic double bonds[1,2,3]. Enhancing lipolysis could decrease biohydrogenation due to the presence of higher levels of PUFA.

Study Aims

To determine the level of PUFA required to inhibit biohydrogenation *in vitro* .

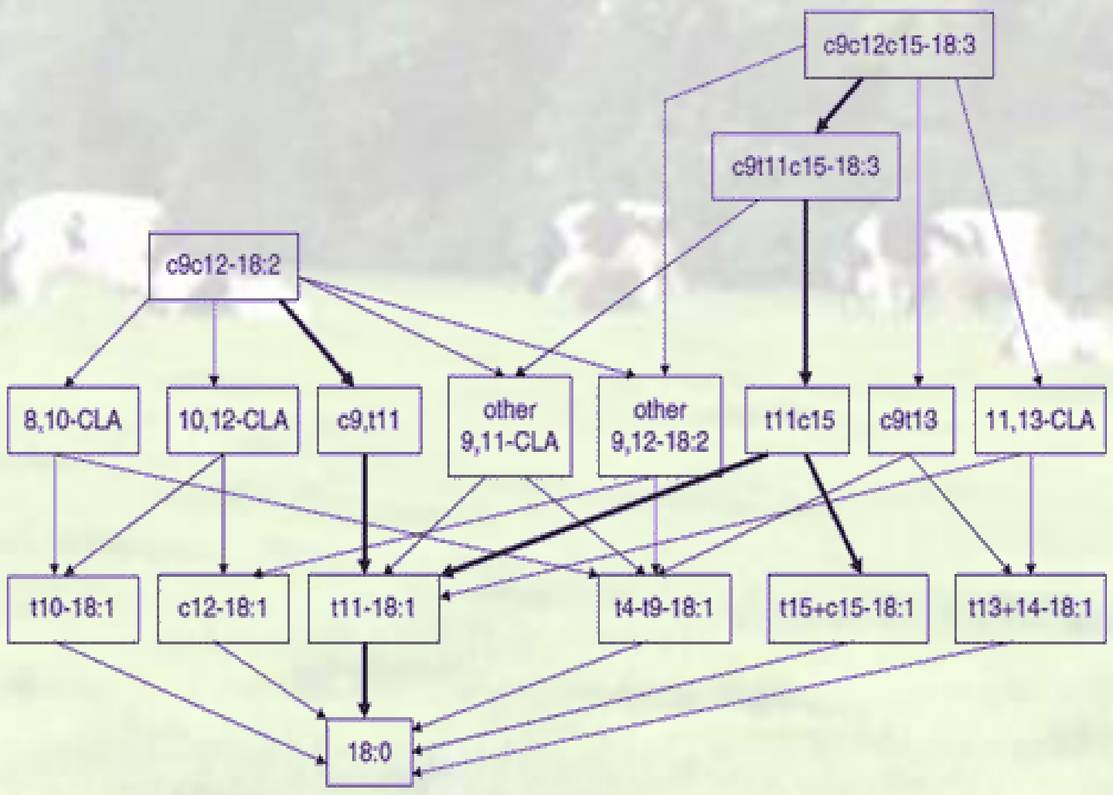

Fig.1: The biohydrogenation process in the rumen, LA-*c*9*c*12-18:2; LNA-*c*9*c*12*c*15-18:3; VA-*t*11-18:1. CLA-conjugated linoleic acid; LA- linoleic acid; LNA- linolenic acid; VA-vaccenic acid[2].

Materials and Methods

A batch culture experiment with 6 treatments was used, each with a basal level of freeze-dried silage and: control (No LA/LNA) or LA/LNA at 50µM and 250µM, 500µM, 750um or 1µM concentrations. These were incubated under rumen like conditions[4] and destructively harvested in triplicate at four time points (0, 4, 8 and 24h). Fatty acid profiles were assessed using FAME analysis and gas chromatography. RNA was extracted, reverse transcribed and a restriction digest carried out using HaeIII before TRFLP to assess the effects on the rumen microbiota.

Acknowledgements

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Results

- LNA and LA at 250µM caused a significant decrease in 18:0 at 4 to 8 h (P= 0.013) of 16.7 and 11.5mg/g-1 (P=0.067) respectively (Table 1 & 2).
- LA and LNA also caused significant increases in 18:3n-3 at 4 hours (increase of 1.98mg/g^{-1}), and changes in 18:2n-6 were significant (P=>0.05) at 4-8 hours (up to 2.12mg/g^{-1}) (Table 1 & 2).
- Concentrations of 250µM and 500µM LA/LNA were most effective in reducing 18:0 and increasing PUFA content (Table 1 & 2).
- TRFLP dendrograms showed no significant changes in the bacterial population relative to time or LA/LNA concentration (data not shown).

Table 1. Effects of linoleic acid addition on fatty acid concentrations *in vitro*

		linoleic acid							
Time	Fatty Acid	Concentration (µM)						SED	P
		0	50	250	500	750	1000		
	18:0	41.1[a]	54.8[a]	52.3[a]	48.1[a]	37.5[a]	41.9[a]	8.44	0.240
	18:1 trans-11	5.96[b]	4.84[a]	4.95[a]	4.77[a]	4.13[a]	4.48[a]	0.51	0.037
0 hrs	18:2 trans-9, trans-11	0.75[a]	0.64[a]	0.69[a]	0.51[a]	1.37[a]	0.88[a]	0.47	0.451
	18:2 cis-9, cis-12	0.01[a]	0.02[a]	0.01[a]	0.01[a]	0.02[a]	0.00[a]	0.01	0.537
	18:3 n-3	0.29[a]	0.02[a]	0.00[a]	0.03[a]	0.44[a]	0.07[a]	0.23	0.273
	18:0	54.7[b]	49.9[b]	40.8[a]	43.0[a]	50.6[b]	49.7[b]	4.40	0.067
	18:1 trans-11	3.94[b]	3.91[b]	3.65[b]	3.36[a]	3.75[b]	4.16[b]	0.25	0.087
4 hrs	18:2 trans-9, trans-11	0.09[b]	0.08[b]	0.06[a]	0.07[b]	0.08[b]	0.09[b]	0.01	0.133
	18:2 cis-9, cis-12	0.96[a]	0.95[a]	2.13[c]	1.64[c]	1.15[b]	0.77[a]	0.26	0.001
	18:3 n-3	1.35[b]	1.19[a]	2.27[c]	1.73[b]	1.41[b]	1.06[a]	0.20	<.001
	18:0	51.1[a]	50.8[a]	44.8[a]	49.0[a]	43.9[a]	52.1[a]	6.47	0.723
	18:1 trans-11	4.22[b]	4.43[b]	3.75[b]	3.51[a]	4.17[b]	4.81[b]	0.50	0.190
8 hrs	18:2 trans-9, trans-11	0.07[a]	0.07[a]	0.05[a]	0.05[a]	0.04[a]	0.03[a]	0.02	0.630
	18:2 cis-9, cis-12	0.67[a]	0.68[a]	0.72[a]	0.46[a]	0.68[a]	0.49[a]	0.12	0.198
	18:3 n-3	1.01[b]	0.97[b]	0.96[b]	0.67[a]	0.84[b]	0.73[a]	0.09	0.015
	18:0	58.2[a]	55.0[a]	56.0[a]	55.8[a]	50.7[a]	61.0[a]	5.94	0.656
	18:1 trans-11	4.20[a]	5.16[a]	4.87[a]	5.15[a]	4.90[a]	3.98[a]	0.94	0.723
24 hrs	18:2 trans-9, trans-11	0.05[b]	0.07[b]	0.05[b]	0.03[a]	0.03[a]	0.06[b]	0.01	0.022
	18:2 cis-9, cis-12	0.61[a]	0.52[a]	1.04[b]	0.53[a]	0.55[a]	0.37[a]	0.13	0.005
	18:3 n-3	0.78[c]	0.72[c]	0.75[c]	0.60[a]	0.58[a]	0.63[b]	0.05	0.006

Table 2. Effects of linolenic acid addition on fatty acid concentrations *in vitro*

		linolenic acid							
Time	Fatty Acid	Concentration (µM)						SED	P
		0	50	250	500	750	1000		
	18:0	54.9[a]	59.0[a]	60.8[a]	51.6[a]	52.5[a]	60.9[a]	5.20	0.335
	18:1 trans-11	4.16[a]	3.86[a]	4.13[a]	6.65[b]	4.08[a]	4.56[a]	0.51	0.001
0 hrs	18:2 trans-9, trans-11	0.06[a]	0.06[a]	0.05[a]	0.07[a]	0.06[a]	0.07[a]	0.01	0.418
	18:2 cis-9, cis-12	0.61[ab]	0.38[a]	0.83[bc]	1.05[c]	0.72[abc]	0.52[ab]	0.19	0.047
	18:3 n-3	0.66[a]	0.48[a]	0.67[a]	1.45[b]	0.74[a]	0.70[a]	0.32	0.123
	18:0	58.9[b]	49.8[ab]	44.1[a]	41.6[a]	48.7[ab]	51.9[ab]	6.19	0.162
	18:1 trans-11	5.48[ab]	5.52[ab]	7.61[b]	6.33[ab]	5.55[ab]	3.64[a]	1.30	0.151
4 hrs	18:2 trans-9, trans-11	0.06[bc]	0.08[c]	0.07[bc]	0.02[a]	0.06[bc]	0.03[ab]	0.02	0.021
	18:2 cis-9, cis-12	0.53[a]	1.580[a]	0.76[a]	0.80[a]	1.28a	0.57[a]	0.54	0.360
	18:3 n-3	0.70[a]	1.51[b]	0.91[abc]	0.84[a]	0.90[ab]	0.64[a]	0.27	0.075
	18:0	57.0[b]	53.8[b]	42.9[a]	50.1[ab]	55.6[b]	57.4[b]	3.61	0.013
	18:1 trans-11	3.54[a]	3.37[a]	3.89[a]	3.98[a]	4.84[a]	3.59[a]	0.71	0.402
8 hrs	18:2 trans-9, trans-11	0.05[b]	0.05[b]	0.00[a]	0.07[c]	0.08[c]	0.08[c]	0.01	<.001
	18:2 cis-9, cis-12	0.44[ab]	0.42[a]	0.64[abc]	1.13[ac]	0.44[ab]	0.43[a]	0.29	0.185
	18:3 n-3	0.76[a]	0.71a	0.80[a]	1.46[b]	0.67[a]	0.67[a]	0.29	0.116
	18:0	54.9[a]	59.2[a]	57.8[a]	55.2[a]	49.4[a]	49.4[a]	4.80	0.263
	18:1 trans-11	3.43[a]	3.65[a]	4.05[a]	4.47[a]	4.56[a]	4.12[a]	0.67	0.531
24 hrs	18:2 trans-9, trans-11	0.06[a]	0.05[a]	0.05[a]	0.06[a]	0.04[a]	0.06[a]	0.02	0.761
	18:2 cis-9, cis-12	0.41[a]	0.46[a]	0.49[a]	1.26[b]	0.50[a]	0.47[a]	0.14	<.001
	18:3 n-3	0.55[ab]	0.52[ab]	0.57[ab]	0.64[b]	0.50[ab]	0.46[a]	0.07	0.243

Discussion

Observed increases in PUFA, and decreases in 18:0 suggest that biohydrogenation was successfully inhibited, particularly when using 250-500µM concentrations. However, after 24 hours biohydrogenating bacteria appear to have recovered, and were biohydrogenating PUFA. RNA analysis showed no changes in the bacterial community over time or concentration, suggesting that rumen function would be unaffected. The next step is to evaluate whether such concentrations are achievable by enhancing lipolysis.